IN VITRO STUDIES ON THE FUNGISTATIC EFFECT OF ANTIHISTAMINIC DRUGS*

WILLSON J. FAHLBERG, PH.D.

The antihistaminic drugs have been shown to be of some value in the therapy of cutaneous diseases. In 1950, Carson and Campbell (1) at the Army Medical School, reported that several cases of tinea pedis (athlete's foot) responded well to the local application of a cream containing two percent Pyribenzamine. On the basis of this observation, the authors undertook a study to determine whether the effect noted was solely against the allergic manifestations incited by the fungi causing the disease or against the fungi themselves. Their results suggested that di-phenyl-pyraline and Pyribenzamine possessed fungistatic activity. Landis and Krop (2) studied the influence of histamine upon the fungistatic action of various antihistamines. They were able to show that the fungistatic action was prevented by suitable amounts of histamine, and the authors established the minimum fungistatic levels for a number of the antihistaminic drugs. Other investigators (3, 4, 5) have reported both *in vitro* and *in vivo* studies on certain of the antihistamines.

These reports suggested the investigation of the relative fungistatic action *in vitro* of a group of the principal antihistaminic agents.

MATERIALS AND METHODS

Antihistamines

The eight antihistaminic compounds studied were as follows: (1) Benadryl, (diphenhydramine hydrochloride) Parke-Davis; (2) Thenylene, (methapyrilene hydrochloride) and (3) Di-Paralene, (chlorocyclizine hydrochloride) Abbott; (4) Neo-Antergan, (pyriamine maleate) Merck; (5) Diatrine, (methaphenilene hydrochloride) Wm. R. Warner; (6) Thephorin, (phenindamine tartarate) Hoffman-La Roche; (7) Pyribenzamine, (tripelennamine hydrochloride) Ciba; (8) Theophylline Ethylenediamine, (aminophylline) Lederle.†

Fungous cultures

The fungi employed were transplants of pure cultures all isolated from clinical cases of superficial or systemic mycoses in man. They had been maintained in the laboratory on Sabouraud's maltose agar. The species of dermatophytes included *Trichophyton Schoenleini*, *Trichophyton tonsurans*, *Trichophyton violaceum*,

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* From the Department of Microbiology, Baylor University College of Medicine, Houston, Texas.

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Trichophyton mentagrophytes, *Trichophyton concentricum*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Hormodendrum compactum*, *Hormodendrum Pedrosoi*, *Monosporium apiospermum*, *Phialophora verrucosa*, *Microsporum canis*, *Microsporum Audouini*, and *Microsporum gypseum*. The fungi causing systemic infections employed were *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Candida albicans*, *Cryptococcus neoformans* and *Sporotrichum Schenckii*.

Procedure

Tubes of Sabouraud's maltose broth were made up in 2.0 ml amounts, each containing 0.1 mg, 0.25 mg, 0.5 mg, 0.75 mg, 1.0 mg, 2.0 mg, 3.0 mg, and 5.0 mg per milliliter, respectively, of the various antihistamines. The pH values of the various solutions were measured after autoclaving.

All of the test organisms were grown on Sabouraud's maltose agar slants, incubated for four weeks at room temperature. The growth was removed aseptically, taking as little of the agar as possible, and placed in a sterile tube containing glass beads and sterile saline. The tubes were then mechanically shaken for 30 minutes, and 0.05 ml of this suspension was inoculated into the series of broth tubes containing increasing concentrations of the antihistaminic drugs, as described above. The tubes were kept at room temperature (approximately 28°C) for 30 days and readings were made at four day intervals. The growth in the test cultures was recorded as one to four plus on the basis of comparison with that in the control broth tubes. All experiments were performed in triplicate.

Of the Sabouraud's maltose broth used for the controls, one portion was adjusted to a pH of 5.8 and the other to a pH of 4.8. These pH values represented the extreme ranges of pH variation observed in the broth containing the antihistamine compounds. Controls were all run in triplicate.

A disc technic similar to that outlined by Carson and Campbell (1) was employed to test the effectiveness of the antihistamine in the presence of serum; the only modification being the use of sterile one-half inch antibiotic discs for the antihistamines in lieu of the penicylinders.

To determine whether the activity of the antihistamines was fungicidal or fungistatic in nature, the Golden and Oster (6) technic as modified by Walker, et al. (4) was followed.

RESULTS

In recording the results of the antihistamine-broth cultures, restriction was considered to have taken place if a comparison with the control tubes indicated substantially less growth in thirty days. Inhibition was recorded if there was no evidence of growth at the end of thirty days.

As shown in Table I, Neo-Antergan, Theophylline, Thenylene, and Pyribenzamine generally exhibited no inhibitory action at concentrations up to 1.0 mg/ml. However, both restriction and inhibition of growth were evident at levels of 2.0 and 5.0 mg/ml with Pyribenzamine and Thenylene. Neo-Antergan and Theophylline, with rare exceptions, were unable to inhibit the growth of most of the fungi tested, even at levels of 5.0 mg/ml, although restriction did take place.

Table II indicates that Benadryl restriectd the growth of most of the fungi

tested at levels of 1.0 mg/ml and Thephorin restricted the growth of all of the fungi tested at levels of 0.25 to 5.0 mg/ml. Both antihistamines were able to inhibit the majority of the fungi tested at the 2.0 and 5.0 mg/ml level. The one notable exception was *C. albicans*, which was not inhibited by either drug at the 5.0 mg/ml level. Diatrine at fairly low levels displayed an ability to restrict all of the fungi tested. The restriction was most marked the first eight days of cultivation and became progressively less. The inhibitory ability of the drug closely paralleled that of Thephorin with the two yeast cultures (*C. neoformans* and *C. albicans*) tolerating a high level of drug. The most effective antihistamine drug of the eight tested proved to be Di-Paralene. Drug levels as low as 0.25 mg/ml

TABLE I

The in vitro *fungistatic activity of pyribenzamine, thenylene, neo-antergan, and theophylline*

FUNGI	PYRIBENZAMINE		THENYLENE		NEO-ANTERGAN		THEOPHYLLINE	
	R*	I*	R*	I*	R*	I*	R*	I*
T. Schoenleini	>1.0	>0.1	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
T. tonsurans	>1.0	>1.0	>1.0	>1.0	1.0	>1.0	>1.0	>1.0
T. violaceum	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
T. mentagrophytes	>1.0	2.0	2.0	5.0	2.0	>5.0	>5.0	>5.0
M. canis	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
M. Audouini	>1.0	2.0	2.0	5.0	1.0	5.0	2.0	5.0
M. apiospermum	2.0	5.0	2.0	5.0	5.0	>5.0	>5.0	>5.0
M. gypseum	2.0	5.0	2.0	5.0	5.0	>5.0	5.0	>5.0
T. concentricum	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
T. rubrum	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
P. verrucosa	2.0	5.0	1.0	5.0	5.0	>5.0	>5.0	>5.0
H. compactum	>1.0	2.0	1.0	2.0	2.0	5.0	1.0	2.0
H. Pedrosoi	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
E. floccosum	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
S. Schenkii	2.0	5.0	2.0	5.0	5.0	>5.0	5.0	>5.0
H. capsulatum	>1.0	2.0	2.0	5.0	2.0	5.0	5.0	>5.0
B. dermatitidis	>1.0	>1.0	>1.0	>1.0	1.0	>1.0	1.0	>1.0
C. albicans	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0
C. neoformans	2.0	5.0	2.0	5.0	2.0	>5.0	5.0	>5.0

* R = mg of drug per milliliter required for restriction of growth.
I = mg of drug per milliliter required for complete inhibition of growth.

were restrictive for the majority of the fungi. Complete inhibitior was evidenced at levels of 0.5 to 0.75 mg/ml. The one exception was *C. neoformans*, which required 2.0 mg/ml for complete inhibition.

To test the effect of the presence of serum in Sabouraud's medium the procedure outlined by Carson and Campbell (1) was followed. The organism first employed was *C. albicans*. Following a heavy inoculation of the plate with the test organism, sterile one half inch antibiotic discs containing known amounts of the various antihistamines were placed on the surface. Diameters of the zones of inhibition were measured at 72 hours. No significant differences were found between duplicate plates.

Table III indicates that Thenylene, Pyribenzamine, Neo-Antergan, and Theophylline required levels of 10 mg/disc to produce an inhibitory zone of appreciable size. The presence of 10 per cent horse serum slightly enhanced the activity of Thenylene, Pyribenzamine, and Theophylline. On the other hand, the activity of Neo-Antergan was almost completely negated by horse serum. Thephorin, Benadryl, and Diatrine showed at the most slight activity in the absence of horse serum at the 2.5 mg/ml level, but in its presence a definite enhancement of activity was observed. The degree of enhancement was fairly constant regardless of

TABLE II

The in vitro *fungistatic activity of diatrine, di-paralene, benadryl, and thephorin*

FUNGI	DIATRINE MG/ML		DI-PARALENE		BENADRYL		THEPHORIN	
	R*	I*	R*	I*	R*	I*	R*	I*
T. Schoenleini	0.75	>1.0	0.25	0.50	0.75	>1.0	1.0	>1.0
T. tonsurans	0.25	>1.0	0.25	0.75	1.0	>1.0	1.0	>1.0
T. violaceum	1.0	>1.0	0.25	0.50	>1.0	>1.0	0.75	>1.0
T. mentagrophytes	0.25	2.0	0.10	0.25	1.0	2.0	1.0	2.0
M. canis	0.50	>1.0	0.50	0.75	>1.0	>1.0	1.0	>1.0
M. Audouini	0.50	2.0	0.25	0.50	1.0	2.0	1.0	2.0
M. Apiospermum	0.75	2.0	0.25	0.75	1.0	>5.0	1.0	2.0
M. gypseum	0.75	2.0	0.25	0.75	<2.0	2.0	1.0	2.0
T. concentricum	1.0	>1.0	0.25	0.50	1.0	>1.0	1.0	>1.0
T. rubrum	0.50	>1.0	0.10	0.50	1.0	>1.0	0.75	>1.0
P. verrucosa	0.10	0.75	0.25	0.75	0.50	2.0	0.25	0.75
H. compactum	0.25	2.0	0.25	0.50	0.5	2.0	0.75	2.0
H. Pedrosi	0.25	>1.0	0.25	0.50	>1.0	>0.0	1.0	>1.0
E. floccosum	0.75	>1.0	0.10	0.75	>1.0	>1.0	1.0	>1.0
S. Schenkii	1.0	3.0	0.25	0.75	2.0	5.0	0.75	2.0
H. capsulatum	0.50	2.0	0.25	0.50	1.0	5.0	0.75	2.0
B. dermatitidis	0.50	>1.0	0.25	0.50	1.0	>1.0	0.75	>1.0
C. albicans+	Not restricted at 3 mg/ml level		0.25	0.75	>5.0	>5.0	5.0	>5.0
C. neoformans+	2 mg/ml	3 mg/ml	0.75	2.0	0.75	2.0	1.0	2.0

*R = mg of drug per milliliter required for restriction of growth.
I = mg of drug per milliliter required for complete inhibition of growth.
+ Two different strains of these fungi were tested with comparable results.

the amount of the drug placed on the disc in the case of Bendryl and Thephorin. With Diatrine at the level of 10 mg/disc, no difference between zone sizes in the presence or absence of horse serum was observed. Di-Paralene activity showed no change in the presence of horse serum when compared with its controls at levels from 1.0 mg/ml to 10 mg/ml.

Comparable results on the effect of serum were obtained in another series of tests employing *C. neoformans* as the test organism, with the exception that in general considerably higher levels of the antihistamines were required to produce inhibitory zones.

The Walker, et al. (4) modification of the Golden and Oster (6) technic for testing for fungicidal activity was employed to analyze the action of Benadryl, Diatrine, Thephorin, and Di-Paralene. Fifteen-day-old cultures of *A. fumigatus*, *C. albicans*, *M. apiospermum*, *M. Audouini*, *M. gypseum*, *S. Schenkii*, and *T. mentagrophytes* grown on ten per cent horse serum Sabouraud's maltose agar, were used as the test organisms. Two immersion times in the test solution were employed. The first time was a one-minute contact as suggested by Golden and Oster. The results indicated that only *T. mentagrophytes* was killed by Diatrine at the 3 mg/ml level, Thephorin at the 3 mg/ml level and Di-Paralene at the 1 mg/ml level. Bendryl had no effect at the 5 mg/ml level, and the growth of none

TABLE III

ANTIHISTAMINE	DRUG LEVEL	ZONE DIAMETER
	mg.	*cm.*
Thenylene	10	2.0
Thenylene & Horse Serum	10	2.5
Pyribenzamine	10	2.0
Pyribenzamine & Horse Serum	10	2.6
Neo-Antergan	10	2.7
Neo-Antergan & Horse Serum	10	0.2
Theophylline	10	2.3
Theophylline & Horse Serum	10	3.0
Thephorin	2.5	0.2
Thephorin & Horse Serum	2.5	1.2
Benadryl	2.5	0.0
Benadryl & Horse Serum	2.5	2.0
Diatrine	2.5	slight
Diatrine & Horse Serum	2.5	2.0
Di-Paralene	1.0	2.7
Di-Paralene & Horse Serum	1.0	2.8

of the other fungi tested were altered by any of the antihistamines at levels up to 5 mg/ml. A second contact time of five minutes was tried, but in the majority of cases the controls with the 95 per cent ethyl alcohol were inhibited, thus invalidating the experiment. In no instance did the 30 per cent acetone controls inhibit the growth of the fungi.

COMMENT

While some of our results on the fungistatic levels of the various antihistamines are not directly comparable with those obtained by Carson and Campbell or Landis and Krop, it is felt that the differences can be rationalized on the basis of strain variation within the species.

SUMMARY

1. The descending order of effectiveness of the antihistamine as fungistatic agents was as follows: Di-Paralene, Diatrine, Thephorin, Benadryl, Pyribenzamine, Thenylene, Theophylline, and Neo-Antergan.

2. Di-Paralene was the most effective drug tested, having a fungistatic activity in amounts of 0.5 to 0.75 mg/ml for all the fungi tested, except *C. neoformans*, which required 2.0 mg/ml for complete inhibition.

3. The presence of 10 per cent serum in most cases enhanced the fungistatic activity of the drugs. Two exceptions, Neo-Antergan in which the activity was negated and Di-Paralene in which there was no effect, were observed.

4. No clear evidence of fungicidal activity could be shown for even the most active fungistatic antihistamines, i.e., Thephorin, Diatrine, or Di-Paralene.

At the present time, studies on the clinical application of Di-Paralene are being pursued, as well as tests for its effect upon experimental systemic mycoses in laboratory animals.

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